EXHIBIT A
PENDING CLAIMS

1. A method of identifying modulators of AMPK-mediated activation of a nitric oxide synthase enzyme selected from the group consisting of eNOS, nNOS and nNOSμ, comprising the step of testing putative modulators for their ability to increase or decrease phosphorylation of the enzyme, said increase or decrease depending on the calmodulin and calcium ion concentrations.

2. A method according to claim 1, in which the specific phosphorylation of Ser-1177 is assessed in the presence of calcium and calmodulin.

3. A method of identifying modulators of AMPK-mediated inhibition of eNOS, comprising the step of testing a putative modulator for its ability to decrease or increase AMPK-mediated phosphorylation of eNOS in the presence of limiting calcium ions.

4. A method according to claim 3, in which the specific phosphorylation of Thr-495 is assessed.

5. (Amended) A method according to Claim 1, in which one or more of the following activities is additionally assessed:

(a) Effect on smooth muscle contraction;

(b) Effect on inotropic activity of the heart;

(b) Effect on chronotropic activity of the heart; or

(d) Effect on platelet function.

6. (Amended) A method according to Claim 1, in which the modulator is an activator, as herein defined.

7. A method according to Claim 6, in which the activator promotes both glucose metabolism and fatty acid metabolism.

8. (Amended) A method according to Claim 1, in which the modulator is an inhibitor, as herein defined.

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9. (Amended) A method according to Claim 3, in which the modulator acts preferentially on non-neuronal cells.

10. (Amended) A method according to Claim 1, in which the modulator promotes the dephosphorylation of Ser-1177 and inhibits eNOS activity.

11. A method according to Claim 3, in which the modulator promotes the dephosphorylation of Thr-495 and stimulates eNOS activity.

12. (Amended) A method according to Claim 1, in which the modulator promotes phosphorylation of nNOS or nNOSμ at Ser-1417.

13. (Amended) A method according to Claim 1, in which the modulator promotes dephosphorylation of nNOS or nNOSμ at Ser-1417.

14. (New) An antibody directed against eNOS, in which the eNOS is phosphorylated at Ser-1177 or at Thr-495.

15. (New) An antibody according to Claim 14, in which the eNOS is phosphorylated at Ser-1177.

16. (New) An antibody according to Claim 14, in which the eNOS is phosphorylated at Thr-495.

17. (New) An antibody according to Claim 14, in which the antibody is raised against a synthetic phosphopeptide comprising the sequence RIRTQSpFSLQER.

18. (New) An antibody according to Claim 14, in which the antibody is raised against a synthetic phosphopeptide comprising the sequence GITRKKTpFKEVANCV.

19. (New) An antibody according to Claim 14, which is a polyclonal antibody.

20. (New) An antibody according to Claim 14, which is a monoclonal antibody.

21. (New) An antibody according to Claim 14, labelled with a detectable marker.

22. (New) A method of detecting phosphorylation of eNOS, comprising the step of reacting a biological sample containing eNOS with an antibody according to claim 14.

23. (New) A method according to Claim 23, in which Ser-1177 is detected.

24. (New) A method according to Claim 23, in which phosphorylation at Thr-495 is detected.

EXHIBIT C

REPLACEMENT SECTIONS

At page 1, after the title, in the current single-paragraph that constitutes the entire Section of the Application pertaining to the field of the invention and cross-reference to related applications, the additions are as shown:

The present application is a nationalization of International Patent Application PCT/AU99/00968, filed November 05, 1999, which claims priority to Australian Patent Application PP 6976, filed November 06, 1998.

This invention relates to the regulation of the activity of the enzyme nitric oxide synthase, and in particular to regulation of activity of endothelial and neuronal nitric oxide synthases. We have found that the phosphorylation of endothelial and neuronal nitric oxide synthases by several protein kinases, including protein kinase C and the AMP-activated protein kinase, regulates their activity.

At page 1, after the title, in the current single-paragraph that constitutes the entire Section of the Application pertaining to the field of the invention and cross-reference to related applications, the final text is as follows:

The present application is a nationalization of International Patent Application PCT/AU99/00968, filed November 05, 1999, which claims priority to Australian Patent Application PP 6976, filed November 06, 1998.

This invention relates to the regulation of the activity of the enzyme nitric oxide synthase, and in particular to regulation of activity of endothelial and neuronal nitric oxide synthases. We have found that the phosphorylation of endothelial and neuronal nitric oxide synthases by several protein kinases, including protein kinase C and the AMP-activated protein kinase, regulates their activity.

In the Section of the Application that forms the Abstract, the deletions and additions are as shown:

This invention relates to the regulation of the activity of the enzyme nitric oxide synthase, [and in particular to] particularly the regulation of activity of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS and nNOSμ). [According to a first aspect, the] The invention provides a method of identifying modulators of AMPK-mediated activation of eNOS, comprising [the step of] testing putative modulators for their ability to increase or decrease phosphorylation of eNOS depending on the calmodulin and calcium ion concentrations. [In an alternative aspect, the] The invention also provides a method of identifying modulators of AMPK-mediated inhibition of eNOS, comprising [the step of] testing a putative modulator for its ability to decrease or increase AMPK-mediated phosphorylation of eNOS in the presence of limiting calcium ions. Preferably, specific phosphorylation of threonine 495 is assessed. [According to a second aspect, the] The invention further provides a method of identifying modulators that either promote or inhibit phosphorylation of nNOS and nNOSμ at Ser-1417. Compounds that activate the AMP-activated protein kinase are expected to be useful in the treatment of ischemic heart disease by promoting both glucose and fatty acid metabolism, as well as by increasing NOS activity to improve nutrient and oxygen supply to the myocytes and to reduce mechanical activity. These compounds [would] also have utility in the treatment of pulmonary hypertension and in obstructive airways disease.

In the Section of the Application that forms the Abstract, the final text is as follows:

This invention relates to the regulation of the activity of the enzyme nitric oxide synthase, particularly the regulation of activity of endothelial nitric oxide synthase (eNOS) and neuronal nitric oxide synthase (nNOS and nNOSμ). The invention provides a method of identifying modulators of AMPK-mediated activation of eNOS, comprising testing putative modulators for their ability to increase or decrease phosphorylation of eNOS depending on the calmodulin and calcium ion concentrations. The invention also provides a method of identifying modulators of AMPK-mediated inhibition of eNOS, comprising testing a putative modulator for its ability to decrease or increase AMPK-mediated phosphorylation of eNOS in the presence of limiting calcium ions. Preferably, specific phosphorylation of threonine 495 is assessed. The invention further provides a method of identifying modulators that either promote or inhibit phosphorylation of nNOS and nNOSμ at Ser-1417. Compounds that activate the AMP-activated protein kinase are expected to be useful in the treatment of ischemic heart disease by promoting both glucose and fatty acid metabolism, as well as by increasing NOS activity to improve nutrient and oxygen supply to the myocytes and to reduce mechanical activity. These compounds also have utility in the treatment of pulmonary hypertension and in obstructive airways disease.

EXHIBIT B
PENDING CLAIMS

1. A method of identifying modulators of AMPK-mediated activation of a nitric oxide synthase enzyme selected from the group consisting of eNOS, nNOS and nNOSμ, comprising the step of testing putative modulators for their ability to increase or decrease phosphorylation of the enzyme, said increase or decrease depending on the calmodulin and calcium ion concentrations.

2. A method according to claim 1, in which the specific phosphorylation of Ser-1177 is assessed in the presence of calcium and calmodulin.

3. A method of identifying modulators of AMPK-mediated inhibition of eNOS, comprising the step of testing a putative modulator for its ability to decrease or increase AMPK-mediated phosphorylation of eNOS in the presence of limiting calcium ions.

4. A method according to claim 3, in which the specific phosphorylation of Thr-495 is assessed.

5. (Amended) A method according to [any one of Claims 1 to 4] Claim 1, in which one or more of the following activities is additionally assessed:

(a) Effect on smooth muscle contraction;

(b) Effect on inotropic activity of the heart;

(b) Effect on chronotropic activity of the heart; or

(d) Effect on platelet function.

6. (Amended) A method according to [any one of Claims 1 to 5] Claim 1, in which the modulator is an activator, as herein defined.

7. A method according to Claim 6, in which the activator promotes both glucose metabolism and fatty acid metabolism.

8. (Amended) A method according to [any one of Claims 1 to 5] Claim 1, in which the modulator is an inhibitor, as herein defined.

9. (Amended) A method according to [any one of Claims 3 to 8] Claim 3, in which the modulator acts preferentially on non-neuronal cells.

10. (Amended) A method according to Claim 1 [or Claim 2], in which the modulator promotes the dephosphorylation of Ser-1177 and inhibits eNOS activity.

11. A method according to Claim 3, in which the modulator promotes the dephosphorylation of Thr-495 and stimulates eNOS activity.

12. (Amended) A method according to Claim 1 [or Claim 2], in which the modulator promotes phosphorylation of nNOS or nNOSμ at Ser-1417.

13. (Amended) A method according to Claim 1 [or Claim 2], in which the modulator promotes dephosphorylation of nNOS or nNOSμ at Ser-1417.

14. (New) An antibody directed against eNOS, in which the eNOS is phosphorylated at Ser-1177 or at Thr-495.

15. (New) An antibody according to Claim 14, in which the eNOS is phosphorylated at Ser-1177.

16. (New) An antibody according to Claim 14, in which the eNOS is phosphorylated at Thr-495.

17. (New) An antibody according to Claim 14, in which the antibody is raised against a synthetic phosphopeptide comprising the sequence RIRTQSpFSLQER.

18. (New) An antibody according to Claim 14, in which the antibody is raised against a synthetic phosphopeptide comprising the sequence GITRKKTpFKEVANCV.

19. (New) An antibody according to Claim 14, which is a polyclonal antibody.

20. (New) An antibody according to Claim 14, which is a monoclonal antibody.

21. (New) An antibody according to Claim 14, labelled with a detectable marker.

22. (New) A method of detecting phosphorylation of eNOS, comprising the step of reacting a biological sample containing eNOS with an antibody according to claim 14.

23. (New) A method according to Claim 23, in which Ser-1177 is detected.

24. (New) A method according to Claim 23, in which phosphorylation at Thr-495 is detected.